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The potential role of blood biomarkers in patients with ischemic stroke: An expert opinion

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Abstract

Blood biomarkers are increasingly beginning to play a role in the diagnosis, management, and prognostication of patients with acute ischemic stroke. While imaging biomarkers have played the largest role in determining acute therapies, blood-based biomarkers may have important contributions to make in settings where imaging is not readily available, or when making predictions about future complications and recurrent stroke. Though more research in large, diverse patient populations are needed before blood-based biomarkers become widely accepted for stroke management, preliminary reports suggest their value in several settings and the use of biomarkers is gaining traction. This article discusses the role of several selected readily available protein biomarkers in stroke diagnosis, acute management decisions, and prognosis. Protein biomarkers were primarily selected based on the fact that they have been evaluated in cohort studies and ideally that they have been validated by independent groups.

Keywords

Biomarker, stroke, outcome, diagnosis, etiology, risk stratification, blood, stroke recurrence

What is a biomarker?

In 1998, a working group at the United States National Institutes of Health defined a biomarker as a biological marker that is objectively measured and evaluated as an indicator of normal biological processes, pathological processes, or pharmacological responses to therapeutic interventions.[1] The term biomarker is thus a generic term that includes physiological parameters, clinical images, and the results of testing of tissue samples, including blood. Surrogate biomarkers are a subset of biomarkers that are used in place of clinical outcomes in trials or other studies. Validated surrogate biomarkers, moreover, include the very limited number of biomarkers for which the evidence of association with clinical disease is considered so strong that the biomarker can substitute for a clinical outcome (such as functional outcome, recurrent stroke, or death) in a clinical trial, and be used to make a determination of approval by a regulatory agency. Examples of validated surrogate biomarkers are generally limited to physiological parameters such as blood pressure or laboratory test results (e.g. viral load) in selected diseases such as human immunodeficiency virus infection. This article focuses on blood protein biomarkers; at present, no blood biomarkers can be considered validated surrogates in the stroke field.

In the setting of acute ischemic stroke, a blood biomarker can be any quantifiable entity that assesses the manifestation of a stroke-related process. The most successful implementation of stroke biomarkers is in areas where information from traditional clinical sources, such as the patient history or clinical examination, is limited. For example, markers can be used to identify patients that may benefit most from acute interventions, reveal stroke etiology, estimate the risk of short-term complications or unfavorable long-term outcomes, and monitor treatment efficacy of secondary prevention.[2] To address these

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Table 1. Selection of protein blood biomarkers in stroke diagnosis.

	Brain tissue injury markers	Inflammatory markers	Hemostatic markers	Miscellaneous markers
Single protein markers	S100B, GFAP, NSE, NMDA-R Ab, MBP, NFls, BNGF	CRP, IL-6, IL-1b, TNF-α, cFn, VCAM 1, MMP9, ApoC-I ApoC-III, chimerin II	DD, vWF, fibrinogen, PAI-1,	BNP or NT-proBNP, PARK 7, NDKA, H-FABP, retinol-binding protein 4, secretatogin, caspase-3, endostatin
Protein marker-based panels	Caspase 3, d-dimer, sRAGE, chimerin II, secretagogin and MMP-9[6]			
	BNGF, MCP-1, MMP-9, S100-B, vWF[7]			
	BNP, DD, MMP9, S100B[8]			
	Eotaxin, MCP-1, S100 A12, MMP-4, prolactin[9]			
	sRAGE, S100B[10]			

ApoC I/III: apolipoprotein-C I/III; BNGF: B-type neurotrophic growth factor; cFN: cellular fibronectin; CRP: C-reactive protein; DD: D-dimer; GFAP: glial fibrillary acidic protein; H-FABP: heart fatty acid binding protein; IL-1b: interleukin-1b; IL-6: interleukin-6; MBP: myelin basic protein; MCP-1: monocyte chemotactic protein-1; MMP-9: matrix metalloproteinases-9; NDKA: nucleoside diphosphate kinase A; Nfl: neurofilament; NMDA-R Ab: *N*-methyl-D-aspartate receptor antibody; NSE: neuron-specific enolase; NT-proBNP: *N*-terminal-pro B-type natriuretic peptide; PAI-1: plasminogen activator inhibitor 1; RBP-4: retinol-binding protein 4; S100-B: calcium binding protein-beta; sRAGE: soluble receptor for advanced glycation end products; TNF-α: tumor necrosis factor-α; VCAM1: vascular cell adhesion molecule 1; vWF: von Willebrand factor.

questions, stroke-related biomarkers may reflect diverse processes immediately preceding stroke or processes during and after stroke.

An effective biomarker should allow precise measurements with a fast turnaround time at reasonable cost.[3] Moreover, it should provide information that is not already available from careful clinical assessment, and its performance should be at least complementary to other available tests.[3] Ideally, a biomarker should assist decision-making and ultimately enhance clinical care.

A biomarker does not necessarily need to be sensitive and specific at the same time, depending on the clinical question being addressed. It might, for example, only be used to rule in a disease (specificity) or rule it out (sensitivity).

To discuss the entirety of different candidate biomarkers that have been studied in stroke patients so far would go well beyond the scope of this article. We aim rather to discuss several interesting candidate protein biomarkers for specific clinical questions that, in our opinion, elucidate concepts of potential implementation in stroke patients.

Diagnostic stroke biomarkers

In the present era of generally available diagnostic neuroimaging, the diagnosis of ischemic stroke is often straightforward. In scenarios in which imaging resources are limited, however, blood-based biomarkers for the diagnosis of stroke may be of value, much like cardiac troponin is used to diagnose cardiac ischemic injury. Also, in prehospital settings a reliable diagnostic biomarker could be helpful to facilitate early diagnosis and triage patients appropriately, since history and exam alone cannot provide a reliable diagnosis.

Among the most important features of a diagnostic biomarker are the ability to determine the presence of a stroke and to differentiate between ischemic and hemorrhagic stroke subtypes, due to diametrically opposed treatments offered for each (e.g. treatment with tissue-type plasminogen activator (tPA) for ischemic stroke versus potential reversal of novel oral anticoagulants in hemorrhagic stroke). Distinguishing ischemic stroke from stroke "mimics," such as migraine, seizure, or hypoglycemia, is also important, although there is evidence that treating mimics with tPA is relatively safe.[4] Thus, the essential measure of a biomarker's utility is whether it can be used to distinguish an ischemic brain lesion from hemorrhagic lesions.

General brain injury biomarkers, such as neuron-specific enolase, are limited in their capacity to serve as markers for ischemic stroke because they are not specific to ischemic stroke, and many disease processes can damage brain tissue. In addition, several brain injury markers are not exclusively released by brain tissue but can also be released by extra-cerebral tissues. Another complicating factor is the fact that the blood–brain barrier restricts the release of these biomarkers into the systemic circulation in the early phase after the injury.

Despite these limitations, several individual biomarkers of brain injury have been evaluated concerning their ability to discern ischemic from hemorrhagic stroke and other mimics.[5] The most commonly studied protein markers of stroke diagnosis are summarized in Table 1. Glial fibrillary acidic protein (GFAP) is one of the most promising and consistent markers. In a multicenter study, GFAP was able to distinguish hemorrhagic from ischemic stroke in the first 6 h after symptom onset with a high sensitivity (81%) and specificity (95%) for the diagnosis of primary hemorrhagic stroke (thus excluding ischemic stroke).[11] A recent independent study was able to confirm these results: A cutoff value of 0.43 ng/mL was the optimal threshold for the differentiation between intracerebral hemorrhage and ischemic stroke, with a sensitivity of 91% and a specificity of 97%.[12] An outstanding question, however, is whether these numbers translate into an acceptable distinction in

the clinical setting in which a choice to use tPA must be made.

The addition of N-terminal brain natriuretic peptide (NT-proBNP) might add incremental value for the differentiation of hemorrhagic versus ischemic strokes. In a recent study including more than 1000 patients into the derivation cohort and more than 700 in an independent validation cohort, among 21 prespecified markers, only NT-proBNP was independently associated with the diagnosis of ischemic stroke when compared to patients with hemorrhagic stroke.[13] Besides NT-proBNP, only endostatin and D-dimer were among those markers with potential for integration into a multimarker panel distinguishing stroke subtypes.[13] Interestingly, all these markers are not brain-specific markers but rather markers associated with the underlying cause of brain ischemia (i.e. etiological markers).

In a preclinical setting (e.g. ambulance), measuring GFAP, NT-proBNP, and potentially other markers with a point-of-care tool could eventually be used to guide ultra-early antihypertensive treatments or rapid reversal of anticoagulation in hemorrhagic stroke patients. In combination with other biomarkers, they might even guide decisions for intravenous tPA treatment where no imaging modalities are available. Currently, however, we are not able to discriminate well enough between stroke subtypes to base most therapeutic decisions on biomarker levels, whether single markers or multimarker panels, and more research is needed.

Prognostic biomarkers after stroke

One of the first questions asked by relatives and patients admitted to the hospital with an acute ischemic stroke relates to their prognosis. Accurate prognosis is not only relevant for patient and relatives but also for physicians to optimize care and allocation of health-care resources. Outcome after ischemic stroke depends on a complex interaction of multiple factors, all of which contribute to break the balance either toward a favorable or unfavorable outcome.[14] Several clinical scales have been developed to predict outcome in stroke patients. One of the best validated and leanest scores contains only two variables—age and the National Institutes of Health Stroke Scale (NIHSS). The score achieved an area under the curve of 0.81 for disability and of 0.71 for mortality 3 months after stroke.[15] Blood markers may increase the prognostic accuracy of scores, keeping them still quite simple and objective at the same time.

Many candidate markers have been studied, and higher levels of biomarkers of most pathophysiological processes involved with stroke are associated with worse outcome after stroke.[16] Even though some markers (e.g. interleukin 6 and NT-pro BNP) do have an independent association with poor outcome, it does not appear that they make very much difference to the accuracy of prediction of poor outcome over and above these more simply measured clinical variables.[17]

One exception may be copeptin, a neuroendocrine marker. Copeptin is released by the hypothalamus in equimolar concentrations to vasopressin, an active hormone involved in the body's response to sympathetic activation and thus stress. Copeptin can therefore serve as a blood biomarker of stress, and its levels have been correlated with the individual stress level in various settings.[18] In a derivation cohort of 362 ischemic stroke patients, the combination of copeptin concentrations with the NIHSS predicted both functional outcome and mortality within 90 days significantly better than the clinical scale or the biomarker alone. The estimated net reclassification improvement for functional outcome was 40% and for mortality 50%, suggesting clinically relevant incremental value for risk stratification.[19] These results were confirmed in several recent independent validation cohorts.[20–22]

Probably even more important than prognosis of overall outcome is an accurate prediction of specific complications after stroke, which then can be directly addressed to improve outcome. Common complications that could be targeted include poststroke pneumonia, seizures, malignant cerebral edema, and hemorrhagic transformation, including in particular symptomatic *intra*cerebral hemorrhage. Few markers have been stringently studied and independently validated in more than one cohort for the prediction of these clinical endpoints, and there is still much work needed.

Matrix metalloproteinase-9 (MMP-9), however, is a promising candidate for the prediction of hemorrhagic transformation and S100B (calcium binding protein-beta) for the development of malignant edema. MMP-9 plasma levels determined prior to thrombolytic therapy have been found to predict hemorrhagic transformation (HT) after intravenous thrombolysis. A graded response was found between pretreatment MMP-9 blood concentrations and the degree of HT.[23,24] Moreover, MMP-9 was also an independent predictor of HT in non-thrombolyzed ischemic stroke patients.[25]

A 12-h S100B value of >0.35 g/L predicted a malignant infarction with a sensitivity of 75% and a specificity of 80%. A 24-h S100B value of >1.03 g/L provided a sensitivity of 94% and a specificity of 83%, though these results have not been independently validated in a larger cohort.[26]

Accurate markers of stroke recurrence also would be very helpful for efficient triage in the emergency setting, especially in patients with transient ischemic attacks (TIAs). So far there have been only a few markers extensively evaluated for this purpose, among them are C-reactive protein (CRP), lipoprotein-associated phospholipase A2 (LpPLA2), and copeptin. CRP has been associated mostly with vascular disease incidence, but some studies also report an association with stroke recurrence,[27,28] while others do not.[29,30] Because CRP is an acute phase reactant, and its concentrations increase in proportion to the severity of the stroke itself as well as the burden of the

patient's comorbidities, CRP tends to correlate more with mortality than stroke risk. CRP has been shown to be helpful for recurrence risk prediction among lacunar stroke patients,[31] however, in whom the burden of brain injury and consequent inflammatory reaction is lower. Another inflammatory marker, Lp-PLA2, metabolizes low-density lipoprotein (LDL) to form free fatty acids and other pro-inflammatory moieties. LpPLA2 was independently associated with mortality but also with risk of recurrent stroke in independent studies.[30,32,33] Two randomized trials of darapladib, a novel inhibitor of Lp-PLA2, have been conducted among patients with cardiovascular disease, but they failed to show that the drug prevents stroke, suggesting that LpPLA2 may not have a direct causal link with stroke risk.[34,35]

Copeptin concentrations have been demonstrated to predict re-events after TIA and stroke, with an incremental value over the usually used clinical risk stratification models, in at least three independent studies.[36–38] Recently, a promising panel of three serum biomarkers—osteopontin, neopterin, and myeloperoxidase—was independently associated with the risk of recurrent stroke, and improved risk classification when added to a clinical risk algorithm with a continuous net reclassification improvement of 29.1%.[39]

These results are promising. The next step would be to compare (ideally in a randomized trial) the clinical gold-standard algorithm for risk stratification including biomarker measurements to the gold standard without the biomarker measurements.

Etiologic stroke biomarkers

Ischemic stroke is a heterogeneous disease. Etiologic classification is specifically important because prognosis, risk of recurrence, and management options differ greatly between etiological subtypes. Considering that up to 30% of stroke patients cannot be classified into a specific subtype,[40] the ability to improve etiological classification to direct prevention methods at the underlying mechanism could be of great value.

The Trial of Organon in Acute Stroke Therapy (TOAST)[41] classification system is the most commonly used in patients with ischemic stroke. This system classifies ischemic strokes as due to large-vessel atherosclerosis, cardioembolic source, small vessel disease, other "determined" causes, and stroke of "undetermined" etiology. The last mentioned category comprises also those patients without known cause due to incomplete evaluation or due to the occurrence of multiple competing causes. Most studies to assess etiological biomarkers have used the TOAST classification system because secondary prevention studies have usually used this system to select patients.

The beginning of the pathophysiological process in ischemic strokes due to large artery atherosclerosis is mainly of an inflammatory nature. Small vessel disease can be attributed to several patho-mechanisms, including similar mechanisms as in large vessel atherosclerosis, but also local fibrinoid necrosis, lipohyalinosis, microatheroma, microaneurysms, and segmental arterial disorganization.[42] As a consequence, blood biomarkers indicating inflammatory vessel processes might be useful etiological biomarker candidates for both large and small vessel diseases. CRP, interleukin-6, interleukin-1b, and tumor necrosis factor alpha all have been implicated in small vessel stroke as well as large vessel strokes.[43–45] In the acute phase after stroke, however, they are also elevated in cardioembolic stroke patients.[46] Moreover, intracellular adhesion molecule-1 (ICAM-1),[47–49] soluble Receptor for Advanced Glycation Endproducts (sRAGE),[50] fibrinogen,[51] P-selectin,[52] and adiponectin[53] have also been associated with large vessel disease. LP-PLA2 was found to be independently associated with ischemic stroke due to large artery atherosclerosis in White non-Hispanic men.[54] In another study,[55] patients suffering from TIA due to large artery atherosclerosis had higher LpPLA2 activity compared to patients suffering from TIA due to non-large artery atherosclerosis.

In the case of cardioembolic stroke, single markers or multimarker biomarker panels indicative of underlying cardiac disease may be most helpful. Among the many evaluated markers, the natriuretic peptides have been studied extensively and the evidence level for these protein markers is currently the highest. Several studies have confirmed that natriuretic peptides, mainly NT-proBNP[55,56] and mid-regional atrial natriuretic peptide (MRproANP),[57] are able to identify primarily cardioembolic stroke subtypes as well as stroke risk.[58] In addition, higher NT-proBNP levels were associated with a relative benefit of warfarin compared with aspirin for prevention of recurrent stroke.[59] Based on these findings, a clinical trial in the United States is testing whether the use of biomarkers, including serum NT-proBNP, among patients with unexplained stroke can be used to select patients with atrial dysfunction ("atrial cardiopathy") for treatment with anticoagulant therapy, just as patients with atrial fibrillation have been treated with anticoagulants for many years (http://clinicaltrials.gov identifier NCT03192215).

Conclusion

The majority of current biomarker studies in vascular neurology have not reached the evidence level to draw final conclusions. However, with over 250,000 proteins, in addition to 20,000 coding genes and an ever increasing number of non-coding genes, metabolites, and lipids, it is important to recognize that the molecular features of human stroke are still being determined and evaluated.[60] Of the many molecules, those with optimal biomarker potential in stroke likely remain at least partly unknown.[60] Efforts to define the molecular fingerprints of stroke are ongoing. However, besides focusing on an improved identification of novel markers and most likely marker panels, future efforts

should also include improvement in validation of interesting candidates, in taking the most promising markers to the next step, and producing evidence of improvement in patient management and ultimately outcome based on blood biomarker guidance.

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